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Characterization of the Two Mutants Y29F and Y29A of Vitreoscilla Hemoglobin

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Vitreoscilla, an obligately aerobic bacterium, synthesizes a relatively large amount of soluble, homodimeric hemoglobin (VHb) under hypoxic conditions. VHb can support aerobic growth in *Escherichia coli* with impaired terminal oxidase function. This ability of VHb to improve the growth properties of *E. coli* has important applications in fermentation technology assisting the over expression of recombinant proteins and antibiotics. The three-dimensional structure of VHb, obtained through x-ray crystallography has shown that the structure confirms to the well-known globin fold. The polypeptide segment connecting helices C and E is disordered and residues E7–E10 do not adopt the usual alpha helical conformation. A key residue in the VHb distal site, Tyr29 (B10), which is highly conserved in various bacterial hemoglobins, may be involved in modulating the oxygen binding properties of VHb. We have introduced two mutations at this site, namely Y29F and Y29A, to see if the side chains of tyrosine (Tyr) have any role in stabilization of oxygen and if any structural change in this position can cause a major change in the functional properties of the protein. The high-resolution crystal structures obtained from these two mutants shows that the Y29F mutant crystallized in the same space group (P21) like that of the wild type with a dimer in the asymmetric unit and the Y29A crystallized in a different space group with the monomer in the asymmetric unit. The missing residues in the disordered region (44, 45, 46, 47, and 50) were built in and showed that the region is helical. In addition, E7 (Q53) is in close contact to the propionate on heme, which is significantly different from the wild type and the Y29F mutant where it faces away. Further studies on these two mutants using the circular dichroism spectra and the carbon monoxide difference spectra has shown that there isn't a major change in the helicity and the binding of heme in the two mutants, which emphasizes that the ordering seen in the Y29A mutant is an artifact of crystallography.